

Isoleucine requirement of 80- to 120-kilogram barrows fed corn-soybean meal or corn-blood cell diets^{1,2,3}

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ABSTRACT: Six experiments were conducted to validate an Ile-deficient diet and determine the Ile requirement of 80- to 120-kg barrows. Experiment 1 had five replications, and Exp. 2 through 6 had four replications per treatment; all pen replicates had four crossbred barrows each (initial BW were 93, 83, 85, 81, 81, and 88 kg, respectively). All dietary additions were on an as-fed basis. In Exp. 1, pigs were fed a corn-soybean meal diet (C-SBM) or a corn-5% blood cell (BC) diet with or without 0.26% supplemental Ile (C-BC or C-BC+Ile) in a 28-d growth assay. On d 14, pigs receiving the C-BC diet were taken off experiment as a result of a severe decrease in ADFI. Growth performance did not differ for pigs fed C-SBM or C-BC + Ile ($P = 0.36$) over the 28-d experiment. In Exp. 2, pigs were fed the C-BC diet containing 0.24, 0.26, 0.28, 0.30, or 0.32% true ileal digestible (TD) Ile for 7 d in an attempt to estimate the Ile requirement using plasma urea N (PUN) as the response variable. Because of incremental increases in ADFI as TD Ile increased, PUN could not be used to estimate the Ile requirement. In Exp. 3, pigs were fed the C-BC diet containing 0.28, 0.30, 0.32, 0.34, or 0.36% TD Ile. Daily gain, ADFI, and G:F increased linearly ($P < 0.01$) as Ile increased in the diet. Even

though there were no effects of TD Ile concentration on 10th rib fat depth or LM area, kilograms of lean increased linearly ($P < 0.01$) as TD Ile level increased. In Exp. 4, pigs were fed a C-SBM diet containing 0.26, 0.31, or 0.36% TD Ile. There were no differences in ADFI or ADG; however, G:F increased linearly ($P = 0.02$), with the response primarily attributable to the 0.31% Ile diet. In Exp. 5, pigs were fed 0.24, 0.27, 0.30, 0.33, or 0.36% TD Ile in a C-SBM diet. There were no differences in growth performance; however, average backfat, total fat, and percentage of fat increased quadratically ($P < 0.10$) with the addition of Ile. In Exp. 6, pigs were fed a 0.26% TD Ile C-SBM diet with or without crystalline Leu and Val to simulate the branched-chain AA balance of a C-BC diet. There were no differences in ADFI or ADG, but G:F increased ($P = 0.09$) when Leu and Val were added. In summary, the Ile deficiency of a C-BC diet can be corrected by the addition of Ile, and because ADFI was affected by Ile addition, the PUN method was not suitable for assessing the Ile requirement. The TD Ile requirement for 80- to 120-kg barrows for maximizing growth performance and kilograms of lean is not $<0.34\%$ in a C-BC diet, but may be as low as 0.24% in a C-SBM diet.

Key Words: Carcass, Isoleucine, Pig, Plasma Urea Nitrogen

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Introduction

Crystalline Lys, Thr, and Trp have become economical for supplementation into many practical swine

diets. To optimize these dietary AA additions, there is a need for an accurate estimation of the fourth-limiting AA, which is Ile according to NRC (1998) in corn-soybean meal (**C-SBM**) diets fed to late-finishing pigs. The NRC (1998) statement is supported by the results of Liu et al. (2000), who reported that a corn diet supplemented with Lys, Thr, Trp, and Met was deficient in Ile. The NRC (1998) requirement for Ile is based on ideal AA ratios (Wang and Fuller, 1989; Chung and

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Baker, 1992; Baker, 1997), but there are few empirical data in the literature estimating the actual true ileal digestible (**TD**) Ile requirement for late-finishing pigs. Early research did not consider the AA digestibility of ingredients, and research was based on small numbers of pigs over a wide weight range (Becker et al., 1963; Brown et al., 1974). More recent reports are inconsistent. Parr et al. (2004) concluded that the TD Ile requirement for 87- to 100-kg barrows was near the NRC (1998) estimate of 0.29% TD Ile for high-lean barrows. In contrast, Kendall et al. (2004) suggested that the TD Ile requirement of 90-kg barrows was 0.36%.

The use of alternative protein sources such as red blood cells, which are deficient in Ile, also created a need to know precisely the Ile requirement and to evaluate the interrelationships of the branched-chain AA (**BCAA**). Recently, Kerr et al. (2004b) in nursery pigs and Parr et al. (2003, 2004) in growing and finishing pigs suggested that a corn-based diet containing 5% red blood cells was deficient in Ile, and that the deficiency could be corrected by the addition of crystalline Ile.

We conducted six experiments to validate an Ile-deficient diet; to determine the TD Ile requirement for high-lean 80- to 120-kg barrows using growth, carcass traits, and minimal plasma area N (**PUN**) as response criteria; and to compare the BCAA relationship of a C-SBM diet to a corn-blood cell (**C-BC**) diet.

Materials and Methods

General

All methods used in these experiments related to animal care were approved by the Louisiana State University (LSU) Agricultural Center Animal Care and Use Committee. Yorkshire, Yorkshire \times Landrace, or Yorkshire \times Landrace \times Duroc pigs from the LSU Agricultural Center were used in each experiment. Pigs were housed in a curtain-sided building with 1.5-m \times 3.0-m pens and concrete slotted floors. Pigs were allotted to dietary treatment on the basis of BW and ancestry in randomized complete block designs. Treatments were replicated five times in Exp. 1 and four times in Exp. 2 through 6; all experiments had four barrows per replicate pen. Diets (Table 1) were formulated on an as-fed basis to meet or exceed the nutrient requirements (with the exception of Ile) of barrows gaining 350 g of lean/d (NRC, 1998) and formulated to contain 0.60% Ca and 0.50% P. Amino acid, mineral, and ME values for corn and SBM were based on NRC (1998). Blood cells (AP 301G; American Protein Corp., St. Louis, MO) were analyzed for total AA content (Table 2), and TD coefficients of 97.1, 95.4, 95.9, and 96.3% were used for Ile, Leu, Val, and Lys, respectively (Parr et al., 2003). Amino acid composition of the blood cells and basal diets was determined after acid hydrolysis (Method 982.30 E[a]; AOAC, 2000). Total sulfur AA content was determined after performic acid oxidation followed by

acid hydrolysis (Method 982.30 E[b]; AOAC, 2000). Tryptophan content was determined after alkaline hydrolysis (Method 982.30 E[c]; AOAC, 2000). Treatment diets in mash form and water were provided ad libitum throughout all experiments. Amino acid additions were made on an as-fed basis.

Experiment 1

Experiment 1 was conducted to validate a C-BC basal diet to be used subsequently in the Ile requirement studies. Sixty barrows with average initial and final BW of 93.3 ± 0.2 and 113.9 ± 2.9 kg were used. The three dietary treatments (Table 1) were 1) C-SBM; 2) C-BC diet containing 5% blood cells; and 3) C-BC with 0.26% supplemental Ile (**C-BC + Ile**).

At the beginning and end of the 28-d growth experiment, 10th rib backfat thickness and LM area were determined by ultrasound (Aloca 500 [12.5-cm and 3.5-MHz probe]; Coremetrics Medical Systems, Wallingford, CT). On d 14, pigs receiving the C-BC diet were removed from the experiment because of a decrease in ADFI.

Experiment 2

Experiment 2 was conducted to estimate the Ile requirement using PUN as the response variable (Knowles et al., 1997). Eighty barrows with average initial and final BW of 82.5 ± 0.8 and 84.3 ± 1.4 kg were used. At the start of the experiment, all pigs received the same late-finishing diet for 3 d and then were bled at 0900 the next day. They were then weighed and allotted to treatment. The C-BC diet containing 0.24% TD Ile (Table 1) was supplemented with increments of 0.02% crystalline L-Ile to provide treatment diets containing 0.24, 0.26, 0.28, 0.30, or 0.32% TD Ile. Pigs remained on treatment diets for 7 d and were bled and weighed at 0900 on d 10.

Blood was collected via the anterior vena cava and placed in 4-mL tubes (Monoject; Sherwood Medical, St. Louis, MO) containing 10.0 mg of sodium fluoride and 8.0 mg of potassium oxalate. Samples were placed on ice before centrifugation at $1,500 \times g$ at 4°C for 20 min. Plasma was collected after centrifugation, and samples were frozen until analysis for PUN by the methods of Laborde et al. (1995).

Experiment 3

Experiment 3 was conducted to estimate the TD Ile requirement using growth performance and carcass traits. Eighty barrows with average initial and final BW of 85.3 ± 0.2 and 117.8 ± 3.6 kg were used. Treatments were the C-BC diet containing 0.28% TD Ile (Table 1) supplemented with increments of 0.02% crystalline L-Ile to provide treatment diets containing 0.28, 0.30, 0.32, 0.34, or 0.36% TD Ile. The growth trial lasted for 32 or 60 d, depending on slaughter date.

Table 1. Composition of basal diets, as-fed basis^a

Ingredients	Exp. 1		Exp. 2		Exp. 3		Exp. 4		Exp. 5		Exp. 6	
	C-SBM 0.47% TD Ile	C-BC 0.24% TD Ile	C-BC 0.24% TD Ile	C-BC 0.28% TD Ile	C-SBM 0.26% TD Ile	C-SBM 0.24% TD Ile	C-SBM 0.26% TD Ile	C-SBM 0.24% TD Ile	C-SBM 0.26% TD Ile	C-SBM 0.24% TD Ile	C-SBM 0.26% TD Ile	
Corn, ground	81.88	90.80	91.27	91.22	94.31	94.82	93.47	94.31	94.82	93.47	93.47	
Soybean meal, 47.5% CP	13.81	—	—	—	1.50	0.47	1.68	1.50	0.47	1.68	1.68	
Spray-dried blood cells ^b	—	5.00	5.00	5.00	—	—	—	—	—	—	—	
Dry fat ^c	0.95	0.30	—	—	—	—	—	—	—	—	—	
Limestone	1.04	1.01	1.01	1.01	1.04	1.04	1.04	1.04	1.04	1.04	1.04	
Monocalcium phosphate	0.84	1.17	1.17	1.17	1.07	1.10	1.08	1.07	1.10	1.08	1.08	
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	
Sodium bentonite	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	
Mineral premix ^d	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	
Vitamin premix ^e	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	
L-Ile	—	—	—	0.04	—	—	—	—	—	—	—	
L-Glu	—	0.24	0.07	0.08	0.08	0.09	0.55	0.08	0.09	0.55	0.55	
L-Lys·HCl	—	—	—	—	0.37	0.40	0.37	0.37	0.40	0.37	0.37	
L-Thr	—	—	—	—	0.09	0.11	0.09	0.09	0.11	0.09	0.09	
L-Trp	—	—	—	—	0.04	0.05	0.04	0.04	0.05	0.04	0.04	
D, L-Met	—	—	—	—	—	0.01	—	—	0.01	—	—	
L-Val	—	—	—	—	—	0.02	—	—	0.02	—	—	
Cornstarch	—	—	—	—	0.02	0.41	0.20	0.02	0.41	0.20	0.20	
Calculated composition ^f												
ME, kcal/kg	3,343	3,343	3,335	3,335	3,293	3,294	3,278	3,293	3,294	3,278	3,278	
CP, %	13.36	12.36	12.24	12.24	9.07	8.70	9.53	9.07	8.70	9.53	9.53	
Lys, %	0.63 (0.62)	0.69 (0.65)	0.69 (0.65)	0.69 (0.66)	0.58 (0.61)	0.58 (0.63)	0.58 (0.60)	0.58 (0.61)	0.58 (0.63)	0.58 (0.60)	0.58 (0.60)	
TSAA, %	0.49 (0.50)	0.41 (0.40)	0.42 (0.40)	0.42 (0.41)	0.36 (0.33)	0.35 (0.35)	0.36 (0.34)	0.36 (0.33)	0.35 (0.35)	0.36 (0.34)	0.36 (0.34)	
Trp, %	0.14 (0.14)	0.12 (0.12)	0.12 (0.12)	0.12 (0.12)	0.11 (0.12)	0.11 (0.13)	0.11 (0.12)	0.11 (0.12)	0.11 (0.13)	0.11 (0.12)	0.11 (0.12)	
Thr, %	0.49 (0.49)	0.47 (0.44)	0.47 (0.44)	0.47 (0.45)	0.39 (0.36)	0.39 (0.42)	0.39 (0.37)	0.39 (0.36)	0.39 (0.42)	0.39 (0.37)	0.39 (0.37)	
Ile, %	0.53 (0.53)	0.27 (0.26)	0.27 (0.26)	0.31 (0.31)	0.30 (0.31)	0.28 (0.29)	0.30 (0.30)	0.30 (0.31)	0.28 (0.29)	0.30 (0.30)	0.30 (0.30)	
Leu, %	1.32 (1.33)	1.55 (1.43)	1.55 (1.44)	1.55 (1.44)	0.99 (0.94)	0.96 (0.97)	0.99 (0.96)	0.99 (0.94)	0.96 (0.97)	0.99 (0.96)	0.99 (0.96)	
Val, %	0.63 (0.64)	0.77 (0.74)	0.77 (0.73)	0.77 (0.74)	0.40 (0.41)	0.40 (0.40)	0.40 (0.42)	0.40 (0.41)	0.40 (0.40)	0.40 (0.42)	0.40 (0.42)	
TD Lys, %	0.54	0.62	0.62	0.62	0.52	0.52	0.52	0.52	0.52	0.52	0.52	
TD Ile, %	0.47	0.24	0.24	0.24	0.26	0.24	0.26	0.26	0.24	0.26	0.26	
TD Val, %	0.55	0.71	0.71	0.71	0.35	0.35	0.35	0.35	0.35	0.35	0.35	
TD Leu, %	1.20	1.45	1.45	1.45	0.91	0.88	0.91	0.91	0.88	0.91	0.91	
Ca, %	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	
P, %	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	

^aC-BC = corn-blood cell, C-SBM = corn-soybean meal, and TD = true digestible.^bAP 301C; American Protein Corp., St. Louis, MO. The analyzed AA values on a percentage as-fed basis were Lys, 8.47; Trp, 1.65; Thr, 3.78; Ile, 0.26; Met + Cys, 1.62; Leu, 12.55; Val, 8.25; Phe + Tyr, 9.17; His, 6.37; and Arg, 3.49.^cFat Pak 100; Milk Specialties Co., Dundee, IL.^dProvided the following per kilogram of diet: Zn, 127 mg; Fe, 127 mg; Mn, 20 mg; Cu, 12.7 mg; I, 0.80 mg; and Se, 0.3 mg as zinc sulfate, ferrous sulfate, manganese sulfate, copper sulfate, calcium iodate, and sodium selenite, respectively, with calcium carbonate as the carrier.^eProvided the following per kilogram of diet: vitamin A, 8,267 IU; vitamin D₃, 2,480 IU; vitamin E, 66 IU; menadione (as menadione pyrimidinol bisulfite complex) 6.2 mg; riboflavin, 10 mg; Ca-D-pantothenic acid, 37 mg; niacin, 66 mg; vitamin B₁₂, 45 µg; D-biotin, 331 µg; folic acid, 2.5 mg; pyridoxine, 3.31 mg; thiamin, 3.31 mg; and vitamin C, 83 µg.^fAmino acid values for corn and soybean meal (NRC, 1998) are on a total and TD basis using digestibility coefficients from NRC (1998). Blood cells were analyzed for total AA values, and TD coefficients of 97.1, 95.4, 95.9, and 96.3% were used for Ile, Leu, Val, and Lys, respectively (Farr et al., 2003). Analyzed values of diets are shown in parentheses.

Table 2. Growth performance, 10th rib fat, and loin muscle area of barrows fed two levels of Ile in a corn-blood cell diet in Exp. 1^a

Item	C-SBM ^b	C-BC 0.24% TD Ile ^b	C-BC 0.50% TD Ile ^b	SEM
Initial BW, kg	93.3	93.3	93.4	0.1
Final BW, kg	112.9	na ^c	114.8	1.3
d 0 to 14				
ADG, kg	0.581 ^d	0.154 ^e	0.701 ^d	0.050
ADFI, kg	2.45 ^d	1.50 ^e	2.37 ^d	0.10
G:F	0.236 ^f	0.101 ^g	0.293 ^h	0.021
d 14 to 28				
ADG, kg	0.820	na	0.831	0.057
ADFI, kg	2.69	na	2.80	0.12
G:F	0.304	na	0.298	0.015
d 0 to 28				
ADG, kg	0.700	na	0.765	0.048
ADFI, kg	2.56	na	2.58	0.10
G:F	0.272	na	0.295	0.010
10th rib fat, cm				
d 0	1.65	1.72	1.70	0.10
d 28	2.08	na	2.11	0.09
LM area, cm ²				
d 0	34.45	36.62	34.71	0.95
d 28	42.09	na	41.48	0.71

^aData are the means of five replicates with four pigs per replicate pen. Data for ADFI and G:F are on an as-fed basis.

^bC-BC = corn-blood cell, C-SBM = corn-soybean meal, and TD = true digestible.

^cna = data not available. On d 14, pigs receiving the C-BC (0.24% Ile) diet were removed from the experiment because of a decrease in ADFI; thus, no data are available for that treatment.

^{d,e}Means within a row with different superscripts differ, $P < 0.001$.

^{f,g,h}Means within a row with different superscripts differ, $P = 0.10$.

Three pigs per pen from the two heaviest replications and three pigs per pen from the two remaining replications were selected randomly and killed by exsanguination after electrical stunning (on d 32 and d 60, respectively) at the LSU Agricultural Center Meats Laboratory. Linear carcass measurements and values from total body electrical conductivity (**TOBEC**; Model MQI-27; Meat Quality Inc., Springfield, IL) were determined as described by Matthews et al. (2001). Fat-free lean and percent fat-free lean were determined by NPPC (2000) equations, which compensate for unequal BW.

Experiment 4

Experiment 4 was conducted to determine whether a C-SBM diet, seemingly deficient in Ile, would respond to dietary supplementation of Ile. Forty-eight barrows with average initial and final BW of 81.0 ± 0.2 and 117.3 ± 3.0 kg were used. Pigs were fed a C-SBM diet containing 0.26% TD Ile (Table 1) supplemented with increments of 0.05% crystalline L-Ile to provide treatments of 0.26, 0.31, or 0.36% TD Ile. The growth trial lasted for 47 d. Pigs and feeders were weighed on d 29 and 47 for calculation of ADFI, ADG, and G:F.

Experiment 5

Experiment 5 was conducted to determine the TD Ile requirement in pigs fed a C-SBM diet. Eighty barrows with average initial and final BW of 80.7 ± 0.2 and 113.0 ± 2.6 kg were used. The basal diet was a C-SBM diet containing 0.24 TD Ile (Table 1), which was subsequently supplemented with increments of 0.03% crystalline L-Ile to provide treatment diets containing 0.24, 0.27, 0.30, 0.33, or 0.36% TD Ile. The growth trial lasted 42 or 49 d, depending on slaughter date. Carcass data were collected as in Exp. 3. Pigs were bled at 0900 via the anterior vena cava on d 42. Handling of the blood and analysis of PUN were the same as in Exp. 2.

Experiment 6

Experiment 6 was conducted to determine the effect of Val + Leu addition to a C-SBM diet. Thirty-two barrows with average initial and final BW of 88.3 ± 0.4 and 113.5 ± 2.8 kg were used. Pigs were fed a C-SBM diet containing 0.26% TD Ile (Table 1) with or without 0.45% crystalline Leu and 0.31% crystalline Val. The addition of Leu + Val resulted in an Ile:Leu + Val that was the same as in the C-BC diet containing 0.28% TD Ile. The growth trial lasted for 38 d.

Statistical Analyses

Data were analyzed by ANOVA procedures using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC) in a randomized complete block design. The statistical model included treatment and replication for all six experiments. Initial PUN was used as a covariate in the analysis of d-10 PUN for Exp. 2. In Exp. 2, 3, 4, and 5, orthogonal contrasts were used to determine linear and quadratic effects of TD Ile. The two-slope NLIN procedure of SAS (break-point analysis) was used to estimate a requirement based on G:F in Exp. 3. Treatment differences were considered significant at $\alpha = 0.10$. The pen of pigs was the experimental unit for all data.

Results

In the diet validation experiment, Exp. 1, pigs receiving the C-BC diet were removed from the experiment on d 14 because of a severe decrease in ADFI, ADG, and G:F ($P < 0.001$; Table 2). Gain:feed was increased ($P = 0.10$) in pigs fed C-BC + Ile at d 14 compared with pigs fed either the C-SBM or C-BC diets. Feed intake, ADG, G:F, 10th rib backfat, and LM area were not different for pigs fed C-SBM or C-BC + Ile for the overall 28-d period.

In Exp. 2 (Table 3), ADFI, ADG, ($P = 0.02$), and G:F ($P = 0.08$) were linearly increased as crystalline TD Ile was increased incrementally from 0.24 to 0.32%. Plasma urea N was not affected by Ile level.

In Exp. 3 (Table 4), final BW, ADG, ADFI, and G:F were increased linearly ($P = 0.01$) as dietary TD Ile

Table 3. Growth performance and plasma urea N concentrations of barrows fed varying levels of Ile in a corn-blood cell diet in Exp. 2^a

Item	True ileal digestible Ile, %					SEM
	0.24	0.26	0.28	0.30	0.32	
Ile intake, g/d	4.61	4.78	5.88	6.93	7.74	—
Ile intake, mg/g of BW gain	25.90	31.66	27.87	22.50	16.06	—
Initial BW, kg	81.8	82.2	82.6	83.5	82.4	0.4
Final BW, kg ^b	83.0	83.2	84.0	85.7	85.8	0.7
Growth performance						
ADG, kg ^b	0.178	0.151	0.211	0.308	0.482	0.101
ADFI, kg ^b	1.92	1.84	2.10	2.31	2.42	0.16
G:F ^c	0.073	0.086	0.095	0.126	0.201	0.049
Plasma						
d 3 Urea N, mmol/L	3.37	2.91	3.48	2.92	3.34	0.22
d 10 Urea N, mmol/L	2.76	2.87	2.79	2.98	2.77	0.21

^aData are the means of four replicates with four pigs per replicate pen. Growth performance was measured from d 3 to 10. Pigs were bled on d 3 and 10, and plasma urea N values from the initial bleed were used as a covariate in the analysis of final plasma urea N. Data for ADFI and G:F are on an as-fed basis.

^bLinear effect, $P = 0.02$.

^cLinear effect, $P = 0.08$.

increased in the diet. There was no significant quadratic effect; however, break-point analysis of G:F estimated the TD Ile requirement at 0.34%. There were no effects of TD Ile level on dressing percent, 10th rib fat depth, average backfat, LM area, or percent muscle; however, carcass length ($P = 0.08$) and kilograms of fat-free lean ($P = 0.002$) were increased linearly as TD Ile level increased (Table 5). Fat-free lean from the TOBEC analysis was increased linearly ($P = 0.002$). There was no effect of TD Ile on TOBEC estimates of total fat, percent lean, or the ratio of lean to fat.

In Exp. 4 (Table 6), addition of Ile to the C-SBM diet containing 0.26% Ile did not affect ADFI or ADG, but G:F was increased linearly ($P = 0.02$), with the response primarily to the 0.31% TD Ile level.

Increasing TD Ile in the C-SBM basal diet (0.24% TD Ile) did not affect final BW, ADFI, ADG, G:F, or PUN (Exp. 5; Table 7). Similarly, Ile supplementation did not affect dressing percent, carcass length, 10th rib fat depth, LM area, fat-free lean, or TOBEC estimates of

kilograms of lean or percent lean (Table 8). Average backfat and TOBEC total fat and percent fat were increased ($P = 0.10$) in a quadratic manner by Ile supplementation. Because fat content increased with no change in leanness, lean:fat decreased as dietary TD Ile increased (quadratic; $P = 0.10$).

In Exp. 6 (Table 9), addition of Leu + Val to a C-SBM diet to create a ratio of Ile:Leu + Val identical to that in the 0.28% TD Ile C-BC diet did not affect final BW, ADFI, or ADG. Gain:feed, however, was increased ($P = 0.09$) when crystalline Leu and Val were added to the diet.

Discussion

The first step in evaluating an AA requirement is to develop a diet that is limiting only in the AA that is of interest. Furthermore, when excess amounts of an AA are supplemented, the diet should support similar rate and efficiency of growth compared with a practical diet.

Table 4. Growth performance of barrows fed varying levels of isoleucine in a corn-blood cell diet in Exp. 3^a

Item	True ileal digestible Ile, %					SEM
	0.28	0.30	0.32	0.34	0.36	
Ile intake, g/d	7.42	8.34	9.28	10.23	11.52	—
Ile intake, mg/g of BW gain	12.51	12.30	12.78	12.90	13.68	—
Initial BW, kg	85.3	85.2	85.3	85.3	85.4	0.1
Final BW, kg ^b	110.5	116.6	118.0	120.6	123.2	1.8
Growth performance						
ADG, kg ^{bc}	0.593	0.678	0.726	0.793	0.842	0.031
ADFI, kg ^{bc}	2.65	2.78	2.90	3.01	3.20	0.09
G:F ^{b,c}	0.223	0.245	0.250	0.263	0.263	0.007

^aData are the means of four replicates with four pigs per replicate pen. Growth performance was measured for 32 or 60 d, depending on processing date. Data for ADFI and G:F are on an as-fed basis.

^bLinear effect, $P < 0.001$.

^cTwo-slope break-point analysis yielded an estimated requirement of 0.34% for G:F.

Table 5. Carcass measurements of barrows fed varying levels of Ile in a corn-blood cell diet in Exp. 3^a

Item	True ileal digestible Ile, %					SEM
	0.28	0.30	0.32	0.34	0.36	
Final BW, kg ^b	110.6	118.5	120.6	120.4	123.2	2.2
Carcass lean and fat measurements						
Loin muscle area, cm ²	46.88	48.01	49.17	47.93	49.80	1.33
10th rib backfat, cm	1.87	1.98	1.96	1.81	2.01	0.13
Average backfat, cm	2.56	2.77	2.71	2.72	2.77	0.10
Carcass length, cm ^c	82.76	82.87	83.82	83.61	84.56	0.71
Dressing percent	74.41	74.23	74.05	73.82	74.93	0.51
Fat-free lean, % ^d	54.54	53.75	53.97	54.30	53.53	0.87
Fat-free lean, kg ^{bd}	44.82	47.24	48.18	48.32	49.50	0.72
Carcass TOBEC analysis using carcass equations ^e						
Fat-free lean, kg ^b	42.14	43.38	44.77	45.63	46.68	0.74
Lean, %	51.31	49.43	50.28	51.34	50.64	0.95
Total fat, kg	23.55	26.61	25.95	24.51	26.66	1.32
Fat, %	28.48	30.07	28.88	27.51	28.84	0.94
Lean:fat	1.83	1.67	1.80	1.89	1.80	0.09

^aData are the means of four replicates with three pigs per replicate pen. TOBEC = total body electrical conductivity.

^bLinear effect, $P = 0.002$.

^cLinear effect, $P = 0.08$.

^dCalculated using the equation for ribbed carcasses described by the NPPC (2000).

^eCalculated using TOBEC analysis with equations from Higbie et al. (2002).

Finally, when considering requirements for finishing pigs, the diet should also result in carcass composition similar to pigs fed a typical diet. In Exp. 1, pigs fed the C-BC diet formulated to be deficient in Ile had decreased ADG and G:F because of a decrease in ADFI. The addition of Ile restored feed intake and growth to a level equal to that of pigs fed the C-SBM positive control. Moreover, evaluations of 10th rib fat thickness and LM area of pigs fed the C-BC diet with supplement

tal Ile did not differ from pigs fed the positive control. The validation of our experimental diet agrees with previous research that suggests a corn-based diet containing 5% red blood cells is deficient in Ile and that it will support optimal growth and carcass composition of 80- to 120- kg barrows when excess Ile is supplied (Parr et al., 2004).

The use of PUN as a response variable has been shown to be a quick and effective means of evaluating

Table 6. Growth performance of barrows fed varying levels of Ile in a corn-soybean meal diet in Exp. 4^a

Item	C-SBM 0.26% TD Ile ^b	C-SBM 0.31% TD Ile ^b	C-SBM 0.36% TD Ile ^b	SEM
Ile intake, g/d	9.00	10.70	12.13	—
Ile intake, mg/g of BW gain	11.87	13.61	15.71	—
Initial BW, kg	81.1	81.0	81.0	0.1
Final BW, kg	116.7	117.9	117.2	1.5
d 0 to 29				
ADG, kg	0.790	0.808	0.789	0.032
ADFI, kg	3.52	3.59	3.41	0.09
G:F	0.224	0.225	0.232	0.004
d 29 to 47				
ADG, kg	0.706	0.752	0.743	0.043
ADFI, kg	3.28	3.23	3.29	0.15
G:F	0.215	0.233	0.225	0.006
d 0 to 47				
ADG, kg	0.758	0.786	0.772	0.032
ADFI, kg	3.46	3.45	3.37	0.11
G:F ^c	0.219	0.228	0.229	0.002

^aData are the means of four replicates with four pigs per pen. Average weight of barrows on d 29 was 104.3 kg. Data for ADFI and G:F are on an as-fed basis.

^bC-SBM = corn-soybean meal, and TD = true digestible.

^cLinear effect of Ile level, $P = 0.02$.

Table 7. Growth performance and plasma urea nitrogen concentrations of barrows fed varying levels of isoleucine in a corn-soybean meal diet in Exp. 5^{a,b}

Item	True ileal digestible Ile, %					SEM
	0.24	0.27	0.30	0.33	0.36	
Ile intake, g/d	6.84	7.72	8.79	10.26	10.58	—
Ile intake, mg/g of BW gain	10.03	10.81	12.72	13.57	15.07	—
Initial BW, kg	80.7	80.6	80.8	80.8	80.8	0.1
Final BW, kg	111.6	113.0	112.2	115.3	112.7	1.3
Growth performance						
ADG, kg	0.682	0.714	0.691	0.756	0.702	0.026
ADFI, kg	2.85	2.86	2.93	3.11	2.94	0.10
G:F	0.241	0.250	0.236	0.243	0.239	0.006
Plasma						
Urea N, mmol/L	1.02	0.93	0.85	1.02	1.04	0.12

^aData are the means of four replicates with four pigs per pen. Growth performance was measured for 42 or 49 d depending on processing date. Data for ADFI and G:F are on an as-fed basis.

^bNo treatment effects, $P > 0.10$.

an AA requirement for pigs at various stages of growth (Knowles et al., 1997; Guzik et al., 2002). The objective of Exp. 2 was to estimate the Ile requirement at the beginning of the 80- to 120- kg growth period by using PUN as the response criteria. However, a linear increase in feed intake led to similar PUN values among pigs fed TD Ile levels from 0.24 to 0.32%. Parr et al. (2004) used feed intake as a covariate in the analysis of PUN and reported results that were inconclusive. Consequently, we believe that PUN is most likely not a good indicator of an AA requirement when feed intakes are different among treatments. Although pigs only received the treatment diets for 7 d in Exp. 2, the magnitude of change in feed intake as Ile increased seemed significant in evaluation of the requirement and led us to increase our range of Ile levels for Exp. 3.

The failure of a requirement estimate from PUN led us to conduct an experiment evaluating the TD Ile requirement of finishing barrows using growth and carcass measurements as response criteria. Again, we observed linear effects of TD Ile on ADFI, and subsequently, ADG and G:F increased as TD Ile increased from 0.28 to 0.36%. Objective requirement estimates based on G:F using two-slope break-point analysis resulted in a requirement of 0.34% TD Ile; however, numerically, ADFI and ADG were greatest for pigs fed the 0.36% TD Ile diet. Our estimate of 0.34% TD Ile is greater than the 0.29% estimate of NRC (1998) and Parr et al. (2004), but it agrees closely with the suggestion of Kendall et al. (2004) that the TD Ile requirement is approximately 0.36%. The carcass responses of an increase in carcass length and kilograms of lean in Exp.

Table 8. Carcass measurements of barrows fed varying levels of Ile in a corn-soybean meal diet in Exp. 5^a

Item	True ileal digestible Ile, %					SEM
	0.24	0.27	0.30	0.33	0.36	
Final BW, kg	113.3	116.0	113.9	116.1	114.3	1.4
Carcass lean and fat measurements						
Loin muscle area, cm ²	43.51	42.28	40.90	43.54	41.24	0.98
10th rib backfat, cm	1.89	1.97	1.85	2.07	1.83	0.11
Average backfat, cm ^b	2.69	3.01	2.86	2.97	2.85	0.08
Carcass length, cm	83.29	83.71	83.40	83.40	83.29	0.54
Dressing percent	74.62	74.24	74.28	74.88	74.59	0.34
Fat-free lean, % ^c	53.32	52.52	52.81	52.22	52.90	0.69
Fat-free lean, kg ^c	45.03	45.19	44.67	45.41	45.09	0.60
Carcass TOBEC analysis using carcass equations ^d						
Fat-free lean, kg	43.61	43.06	43.11	43.58	43.97	0.66
Lean, %	51.71	50.02	50.97	50.15	51.56	0.77
Total fat, kg ^b	24.32	25.91	24.85	26.57	24.15	0.78
Fat, % ^b	28.60	30.08	29.37	30.55	28.33	0.75
Lean:fat ^b	1.87	1.68	1.75	1.65	1.84	0.08

^aData are the means of four replicates with three pigs per pen. TOBEC = total body electrical conductivity.

^bQuadratic effect, $P = 0.10$.

^cCalculated using the equation for ribbed carcasses described by the NPPC (2000).

^dCalculated using TOBEC analysis with equations from Higbie et al. (2002).

Table 9. Growth performance of barrows fed diets with two different ratios of Ile:Leu + Val in Exp. 6^a

Item	C-SBM 0.26% TD Ile ^b	C-SBM + Leu + Val ^b	SEM
Initial BW, kg	88.5	88.0	0.2
Final BW, kg	111.7	115.2	1.4
Growth performance			
ADG, kg	0.610	0.718	0.037
ADFI, kg	2.79	2.93	0.12
G:F ^c	0.218	0.245	0.007

^aData are means of four replicates with four pigs per pen. Data for ADFI and G:F are on an as-fed basis.

^bC-SBM = corn-soybean meal; TD = true ileal digestible.

^cTreatment effect, $P = 0.09$.

3 are likely the result of an increase in final BW caused by increased feed intake and growth. There were no significant differences among measurements of carcass composition when lean and fat were expressed as a percentage of carcass weight.

Our greater-than-expected requirement estimate of 0.34% TD Ile in a C-BC diet suggested that a C-SBM diet formulated with crystalline sources of the first three limiting AA (Lys, Thr, and Trp) should respond to additions of Ile. Experiment 4 was conducted to compare the response of Ile in a C-SBM diet with the response previously observed in a C-BC diet. Gain:feed increased when TD Ile increased from 0.26 to 0.31%, with no further improvement to the 0.36% level. Previous experiments with the C-BC diet indicated we would expect to observe a decrease in ADFI in a diet containing 0.26% TD Ile. Several researchers have observed feed intake responses to dietary supplementation of Ile (Parr et al., 2003; Kendall et al., 2004; Kerr et al., 2004a); however, in Exp. 4, ADFI was numerically greatest with the C-SBM diet that contained 0.26% TD Ile. All pigs had high ADFI (>3.3 kg). The data from this experiment suggested that the TD Ile requirement was between 0.26 and 0.31% in a C-SBM diet.

Experiment 5 was conducted to evaluate more thoroughly the TD Ile requirement of late-finishing barrows fed a C-SBM diet. Data from Exp. 4 indicated that G:F was improved when TD Ile increased from 0.26 to 0.31%. In Exp. 5, there were no significant differences in growth performance when levels of TD Ile ranged from 0.24 to 0.36%. A quadratic response of average backfat and TOBEC estimates of total fat and percent fat seem to be related to differences in the final BW of pigs within level of Ile supplementation. Measures of lean did not differ because of Ile supplementation. The results of this experiment do not agree with the results of Exp. 4, which suggested a TD Ile requirement >0.26% TD Ile for maximizing G:F in a C-SBM diet. The results of this experiment also do not agree with the results of Exp. 3, which suggested a TD Ile requirement of at least 0.34% in pigs fed a C-BC diet.

A review of the literature on the Ile requirement of pigs (Table 10) resulted in eight requirement estimates

from peer-reviewed journal articles (Becker et al., 1963; Oestemer et al., 1973; Taylor et al., 1985; Parr et al., 2003; Kerr et al., 2004a; Parr et al., 2004), four estimates from experiment station reports (Bergstrom et al., 1996; James et al., 2001), and two estimates from abstracts (Lenis and van Diepen, 1997; Kendall et al., 2004). Researchers used various techniques to estimate plateaus and break points in these studies. Thus, to evaluate data from these experiments more consistently, break-point analysis was performed on treatment means to estimate requirements when the authors used some other statistical method. Because many of these experiments were based on total Ile rather than on TD, a digestibility coefficient of 88% was used to calculate a TD Ile value when none was reported or when it was impossible to calculate the value from dietary ingredients. Growth performance values of pigs at or above the estimated requirement were used to calculate Ile intake and the milligrams of Ile required for 1 g of BW gain. The average estimate from Table 10 was 8.97 mg of Ile/g of BW gain. Kerr et al. (2004a) recently reported a similar review of the Ile requirement using some of the same data and had a slightly lower estimate of 8.69 mg of Ile/g of BW gain.

When calculations were made to compare the milligrams of Ile needed per gram of BW gain in our C-SBM diet, the lowest value was 10.03 mg/g for the diet containing 0.24% TD Ile. Pigs fed this diet had growth performance similar to pigs fed higher levels of Ile supplementation, suggesting that 10.03 mg/g was above the requirement. Based on our review of the literature, it does not seem likely that Ile would become limiting in a C-SBM diet for late-finishing barrows unless feed intake is restricted or genetic growth potential is extremely high. An estimate of 8.97 mg of Ile/g of BW gain from our review seems reasonable based on a lack of response to levels above that in our studies with C-SBM diets and estimates from the existing literature (Table 10). Kerr et al. (2002) recently reviewed the Lys requirement and estimated that 17.89 mg of Lys/g of BW gain were needed for optimal performance. With the use of this estimate for the Lys requirement and our review estimate for Ile, the Ile:Lys is 0.50. Based on the feed intake and growth performance data of pigs in Exp. 5, the TD Lys requirement would be 0.43%, with a TD Ile requirement of 0.22%. Without creating a purified diet, there is no known combination of typical feed ingredients, other than red blood cells, that would allow for a response to Ile. In Exp. 3 in the C-BC diet, 12.90 mg of Ile/g of BW gain was needed to obtain optimum performance, which is much higher than was needed in pigs fed the C-SBM diet. Based on the performance of these pigs and the Lys requirement estimate of Kerr et al. (2002), they would have required a diet containing 0.47% TD Lys. Thus, the required ratio of Ile to Lys in a diet containing 5% red blood cells would be 0.72, which seems to be an unreasonably high estimate to apply to diets that do not contain red blood cells.

Table 10. Isoleucine requirement estimates of growing pigs, as-fed basis

BW, kg			Growth performance			Ile				Reference
Mean	Initial	Final	ADG, g	ADFI, g	G:F	Total, % ^a	TD, % ^b	TD, g/d ^c	mg/g of ADG ^d	
7.0	5.6	8.4	236	257	0.92	0.78	0.69	1.77	7.50	James et al. (2001) ^e
7.0	5.6	8.4	212	252	0.84	0.70	0.62	1.56	7.36	James et al. (2001) ^f
8.3	6.6	9.9	255	358	0.71	0.69	0.61	2.18	8.55	Kerr et al. (2004a) ^g
8.4	5.1	11.6	205	340	0.60	0.78	0.69	2.35	11.46	Becker et al. (1963) ^h
8.8	6.6	10.9	313	409	0.77	0.74	0.65	2.66	8.50	Kerr et al. (2004a) ⁱ
10.8	5.8	15.7	385	623	0.62	0.48	0.43	2.68	6.96	Oestemer et al. (1973) ^j
17.5	11.4	23.5	435	972	0.45	0.42	0.37	3.60	8.29	Bergstrom et al. (1996) ^k
17.5	11.4	23.5	578	994	0.58	0.63	0.55	5.47	9.46	Bergstrom et al. (1996) ^l
29.0	18.0	40.0	685	1,250	0.55	0.57	0.50	6.25	9.12	Lenis and van Diepen (1997) ^m
34.7	27.0	42.3	727	1,468	0.50	0.60	0.53	7.78	10.70	Parr et al. (2003) ⁿ
40.0	25.0	55.0	624	1,596	0.39	0.43	0.38	6.06	9.71	Taylor et al. (1985) ^o
52.9	44.6	61.1	600	1,775	0.34	0.39	0.34	6.04	10.07	Becker et al. (1963) ^p
93.0	87.0	99.0	736	1,636	0.45	0.35	0.31	5.07	6.89	Parr et al. (2004) ^q
103.5	91.0	116.0	1,210	3,620	0.34	0.42	0.37	13.39	11.07	Kendall et al. (2004) ^r
									8.97	Overall mean

^aWhen Ile values were not given on a total basis, values were calculated from true ileal digestible (TD) Ile by dividing by 0.88 or from the apparent digestible Ile by dividing by 0.80.

^bWhen possible, digestibility values were taken from the articles. If they were not reported, a digestibility value of 88% was used to estimate TD Ile.

^cCalculated grams of TD Ile consumed per day.

^dCalculated milligrams of Ile required for 1 g of BW gain.

^eJames et al. (2001). Diet contained 1.26% apparent digestible Lys. Break-point analysis requirement estimate for ADG was 0.69% TD Ile.

^fJames et al. (2001). Diet contained 1.00% apparent digestible Lys. Break-point analysis requirement estimates were 0.62, 0.62, and 0.61% TD Ile for ADG, ADFI, and G:F, respectively.

^gKerr et al. (2004a). Results indicated a requirement of 0.69% TD Ile and 9.9 mg of TD Ile/g of BW gain. Break-point analysis estimated the requirement at 0.61% for both ADG and G:F.

^hBecker et al. (1963). Exp. 1; break-point analysis estimated the requirement at 0.68 and 0.70% for ADG and G:F, respectively. The average requirement was 0.69% TD Ile.

ⁱKerr et al. (2004a). Results indicated a requirement of 0.70% TD Ile and 9.1 mg of TD Ile/g of BW gain. Break-point analysis estimated the requirement at 0.67 and 0.62% for ADG and G:F, respectively. The average requirement was 0.65% TD Ile.

^jOestemer et al. (1973). Break-point analysis estimated the requirement at 0.44% for ADG and 0.42% TD Ile for G:F. The average requirement was 0.43% TD Ile.

^kBergstrom et al. (1996). Exp. 2; break-point analysis estimates for pigs fed 0.75% TD Lys were 0.38 and 0.36% TD Ile for ADG and ADFI, respectively.

^lBergstrom et al. (1996). Exp. 2; break-point analysis estimates for pigs fed 1.10% TD Lys were not used. Performance was improved to a level of 0.55% TD Ile, with no subsequent further improvement for ADG or ADFI.

^mLenis and van Diepen (1997). Data taken from Kerr et al. (2004).

ⁿParr et al. (2003). Exp. 2; break-point analysis estimated requirements of 0.55 and 0.51% TD Ile for ADG and ADFI, respectively. The average requirement was 0.53% TD Ile.

^oTaylor et al. (1985). Break-point analysis estimated requirements of 0.41 and 0.34% TD Ile for ADG and G:F, respectively. The average requirement was 0.38% TD Ile.

^pBecker et al. (1963). Exp. 4; break-point analysis estimated requirements of 0.35 and 0.33% TD Ile for ADG and G:F, respectively. Data based on only four pigs per treatment.

^qParr et al. (2004). Exp. 2; break-point analysis estimated the requirement at 0.31% TD Ile for ADG.

^rKendall et al. (2004). Break-point analysis estimated requirements of 0.36, 0.37, and 0.39% TD Ile for ADG, ADFI, and G:F, respectively. The average requirement was 0.37% TD Ile.

Our relatively high estimate of the TD Ile requirement of late-finishing barrows when using the C-BC diet may be a result of the BCAA balance. Red blood cells are extremely high in Leu (12.55%) and Val (8.25%), but comparatively low in Ile (0.26%). Numerous researchers have suggested an antagonism among the BCAA (Tannous et al., 1966; Allen and Baker, 1972; Edmonds and Baker, 1987). Papet et al. (1988) observed that excess Leu increases the activities of branched-chain aminotransferase in the liver and jejunum and the branched-chain keto-acid deaminase in the jejunum in preruminant lambs. Other researchers have suggested that an increased metabolic oxidation of Ile might occur when dietary levels of Leu are high (Tannous et al., 1966; Calvert et al., 1982; Edmonds and

Baker, 1987). Edmonds and Baker (1987) demonstrated that plasma AA concentrations of Ile and Val were decreased when Leu was supplied in excess of 1 to 6%. It may be that the use of a C-BC diet overestimates the Ile requirement because of these metabolic conditions.

The calculated composition of our 0.24% TD Ile C-BC diet contained 0.57% more TD Leu than the 0.24% TD Ile C-SBM diet used in Exp. 4. This resulted in Leu:Ile of 5.58 and 3.51 for the 0.26% TD Ile C-BC and C-SBM diets (Table 11), respectively. Barbour and Latshaw (1992) reported no increase in the Ile requirement of broiler chicks when Leu and Val were increased in the diet; however, their proposed imbalanced diet had a Leu:Ile of 3.6, which is only slightly greater than in the C-SBM diets we used in Exp. 4 and 5. The ratio

Table 11. Branched-chain AA comparison of corn-soybean meal and corn-blood cell diets^{a,b}

Item	C-BC 0.26% TD Ile	C-BC 0.36% TD Ile	C-BC 0.50% TD Ile	C-SBM 0.26% TD Ile	C-SBM 0.36% TD Ile
Ile:Lys ^c	0.42	0.58	0.81	0.50	0.69
Leu:Ile	5.58	4.03	2.90	3.51	2.52
Val:Ile	2.73	1.96	1.41	1.35	0.97
Leu + Val:Ile	8.31	5.99	4.31	4.86	3.49
Ile:Leu + Val	0.12	0.17	0.23	0.21	0.29

^aC-BC = corn-blood cell, C-SBM = corn-soybean meal, and TD = true digestible.

^bLysine and BCAA ratios are calculated from true ileal digestible values.

^cTrue ileal digestible Lys values were 0.62 and 0.52% for the C-BC and C-SBM diets, respectively.

of Leu to Ile in our 0.36% TD Ile C-BC diet was 4.03, which approached the range calculated for our C-SBM diets. The Leu:Ile where performance begins to become impaired and the requirement for Ile increases in the pig is unknown, and it may vary depending on pig weight, genotype, and environment. Thus, it is difficult to determine whether our C-BC diet was responding to a deficiency of Ile or responding to a correction of the BCAA balance.

Experiment 6 was conducted to determine the effect of simulating the BCAA balance of the C-BC diet containing 0.28% TD Ile in a C-SBM diet containing 0.26% TD Ile. The addition of 0.45 and 0.31% crystalline Leu and Val numerically increased feed intake and significantly improved G:F compared with pigs fed the control diet. These results suggest that the ratios of BCAA in previous experiments were not the cause of the decreased ADFI and ADG in pigs fed the C-BC diet. Nonetheless, there are still significant differences in the composition of the C-SBM diet compared with the C-BC diet. The C-SBM diet was approximately 2.7% lower in CP than the C-BC diet. Torres et al. (1998) reported that the CP concentration in diets for rats affected the regulation of branched-chain aminotransferase. The low CP value of the C-SBM diet in Exp. 4, 5, and 6 may limit the activation of branched-chain aminotransferase, and thus, the pig would not be as sensitive to excess levels of Leu and Val. Furthermore, the possibility exists that the crystalline forms of Leu and Val in our C-SBM diet were absorbed at different rates than Leu and Val from intact protein (Yen et al., 2004). Although the efficiency of utilization of crystalline AA in pigs that are fed ad libitum is usually considered equal to intact protein sources (Yen et al., 2004), the differences in absorptive rates may play a role in altering the rate of BCAA oxidation.

In summary, the TD Ile requirement for 80- to 120-kg barrows for maximizing feed intake, growth, feed efficiency, and kilograms of lean is not <0.34% or 12.90 mg of Ile/g of BW gain in a corn diet containing 5% blood cells. In contrast, the requirement may not be >0.24% in finishing barrows fed a C-SBM diet. Neither of these estimates agrees with the NRC (1988) suggestion of 0.29%. In addition, the PUN method is not suitable for assessing the Ile requirement of pigs because

of tremendous changes in ADFI. Our data suggest that Ile is not a limiting nutrient in formulation of low-CP C-SBM diets for late-finishing barrows. Further research is needed to evaluate the optimal BCAA balance and the maximum level of red blood cells that can be fed to pigs.

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